# PRELIMINARY STUDIES ON CHITIN DECOMPOSITION IN LAKE ERIE SEDIMENTS[1]

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*Abstract.* Enrichment studies, biproduct analyses, and slides coated with chitin were employed to examine chitin decomposition in Lake Erie sediments and the water-sediment interface. Enumeration studies of sediment indicated the presence of approximately $10^6$ $ml^{-1}$ heterotrophic bacteria of which approximately 10% ($10^5$ $ml^{-1}$) displayed chitinolytic ability. Chitin-coated slides buried in a mud jar gave preliminary evidence of a succession of microorganisms active in chitin degradation in both the aerobic and anaerobic zones of the sediment column. Enrichment studies of frozen core sections showed the potential activity of microorganisms in the sediments to degrade chitin with the release of $NH_3$. Tests for release of the subunit of chitin, N-acetylglucosamine, from enriched sediments were not successful.

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The process of degradation within an aquatic ecosystem plays an important role in the cycling of nutrients within that ecosystem. Chitin decomposition is significant in light of the large quantity of chitin present in the aquatic ecosystem and its relatively high nitrogen content. The release of the organically bound nitrogen as well as carbon, is a substantial factor to be considered in carbon and nitrogen cycling in the ecosystem.

The primary means of chitin decomposition in the aquatic environment is bacterial activity. A number of review articles have been published on microbial chitin decomposition (Hood and Meyers 1973; Campbell and Williams 1951; Benton 1935). Bacteria responsible for this activity have been isolated and identified by Veldkamp (1955), Campbell and Williams (1951), and Zobell and Rittenberg (1938), to name a few. Enumeration of such organisms, however, has been limited. Zobell and Rittenberg (1938) attempted enumeration of organisms in marine sediments, with little success. Skinner and Davis (1937) and Veldkamp (1955) have enumerated chitinoclasts in soils. Few attempts, however, have been made with fresh water sediments. Okutani (1975) enumerated chitinoclasts in the sediment and water column from Lake Biwa, a freshwater lake in Japan. The present paper describes potential microbial communities active in chitin degradation within the sediment and sediment-water interface of the western basin of Lake Erie.

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## MATERIALS AND METHODS

*Collection and Storage of Sediment.* Sediment cores for examination were collected approximately 30 m north of Rattlesnake Island in 6.1 m of water in the Bass Islands region of western Lake Erie during October 1973. All cores were taken manually using a brass corer into which butyrate tubing with dimensions 1⅜"ID x 1½"OD was inserted. Cores were stored in an ice chest in transport (3 hours) and immediately placed in −20°C freezer until needed.

Sediment for the mud jar experiment was taken with an Ekman dredge and stored in an ice chest until returned to the lab. The mud was placed in a 4 liter beaker and allowed to equilibrate at room temperature to simulate *in situ* conditions.

*Enumeration Medium and Inocula.*

The basic chitin mineral salts medium used was that described by Skerman (1967) for isolation of chitinolytic organisms capable of using chitin as a sole source of carbon and nitrogen. The method for enumeration required plates prepared by the overlay technique. Chitin purchased from Calbiochem (Los Angeles, CA) was used for all tests after treatment by washing and reprecipitation as outlined by Skerman (1967). Total heterotrophic counts were made

using PCA (Difco) medium. Mud inocula for enrichment and enumeration studies were taken from 2 cm sections of the sediment core after slicing the frozen core and allowing it to thaw. Enumeration of potentially chitinolytic bacteria in mud sediment was determined by serial dilution of mud sediment from varying depths. A positive test for chitinase activity was indicated by a zone of clearing surrounding a colony on chitin agar. All platings were carried out in duplicate. Total heterotrophic counts were determined using plate counts on PCA, conducted in duplicate. Plates were incubated both anaerobically, in 95:5 mixture of $CO_2$ and $N_2$, and aerobically at 22°C. Plates were counted after 48 hr for PCA and after 12 days for chitin agar, due to the slow growth on this limited medium.

*Detection of $NH_3$ and N-acetylglucosamine (NAGA).* Test tubes containing 8 ml of mineral salts media and 1 ml reprecipitated chitin (4.57 mg) were inoculated with approximately 1 ml sediment from a particular section of the core. Tubes containing 9 ml mineral salts media with 1 ml sediment were used as controls of unenriched sediments. These procedures were done in triplicate with tubes being placed in both aerobic and anaerobic environments at 22°C. Positive results were noted after 14 days incubation, at which time the supernatants were tested for $NH_3$, by Nesslerization (Amer. Public Health Assoc. 1971), and NAGA, by the method of Morgan and Elson (1934). Control values were substracted from the average of the 3 tubes and net values were used for graphical purposes.

*Buried Slide Technique.* To test for chitinolytic organisms in the mud, acid and alcohol cleaned slides were coated with reprecipitated chitin. Heat fixing the coated slide permitted the slides to be placed in the mud jar without losing the chitin film. The method used was modified from that of Tribe (1957). The mud in the jar was covered with approximately 2 cm of water and kept at 22°C. Slides were removed at various time intervals (days), washed with tap water to remove dirt particles, stained with safranin, examined, and photographed at a magnification of 400X. Observations of morphological types and degree of hydrolysis of chitin at various time periods and sediment depths were recorded.

## RESULTS

Potentially chitinolytic organisms proved to be from 1 to 10% of the total

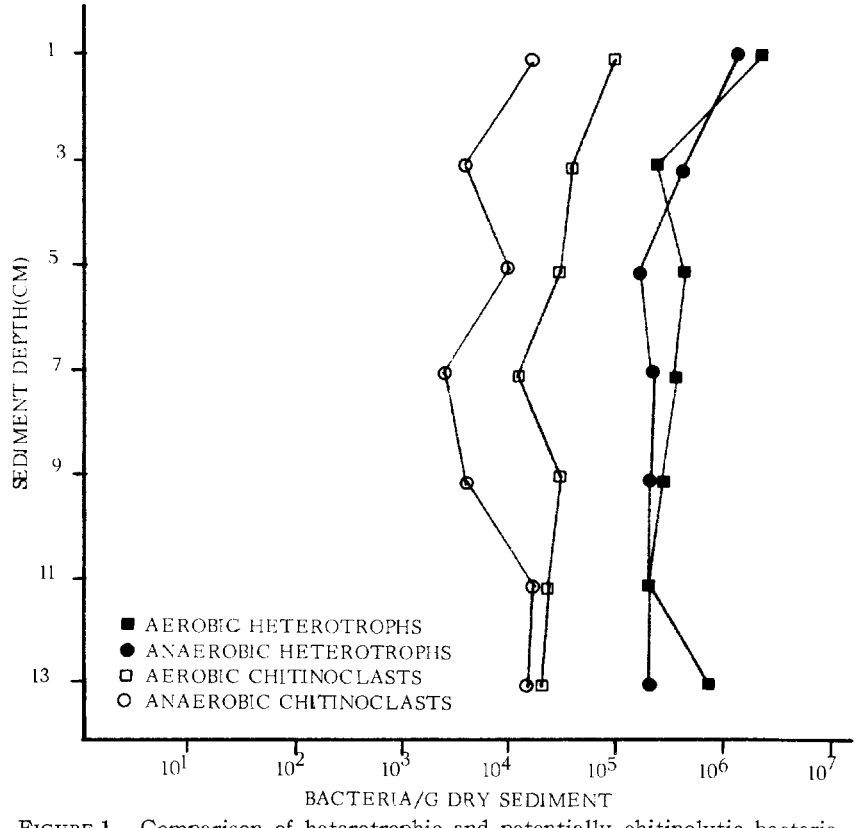


FIGURE 1. Comparison of heterotrophic and potentially chitinolytic bacteria within sediment after 2 day (solid circles and squares) and 12 day (open circles and squares) incubation at 22°C.

heterotrophic population in sediments. Figure 1 shows the heterotrophic and chitinolytic organisms found at the various depths. Very little detectable $NH_3$ or NAGA was noted following 4 days incubation of mud enriched with chitin. However, after 14 days there was an increase in $NH_3$ production above controls without chitin. With 2 exceptions, aerobic $NH_3$ production exceeded detectable anaerobic production (fig. 2). After this period of time nearly all samples showed a complete loss of visible reprecipitated chitin. Attempts to detect NAGA were unsuccessful after both 4 and 14 days in all instances.

Control slides with no chitin film were examined to determine bacterial flora associated with the glass at the water-mud interface typical fields were studied on these slides after 7 day incubation at 22°C (fig. 3). Bacterial spores and fungal and algal filaments were seen. Clusters of bacterial cells indicating bacterial growth were not noted. Figure 4 shows what is believed to be chitin hydrolysis in the aerobic zone of the mud. The slide was removed from incubation after 6 days at

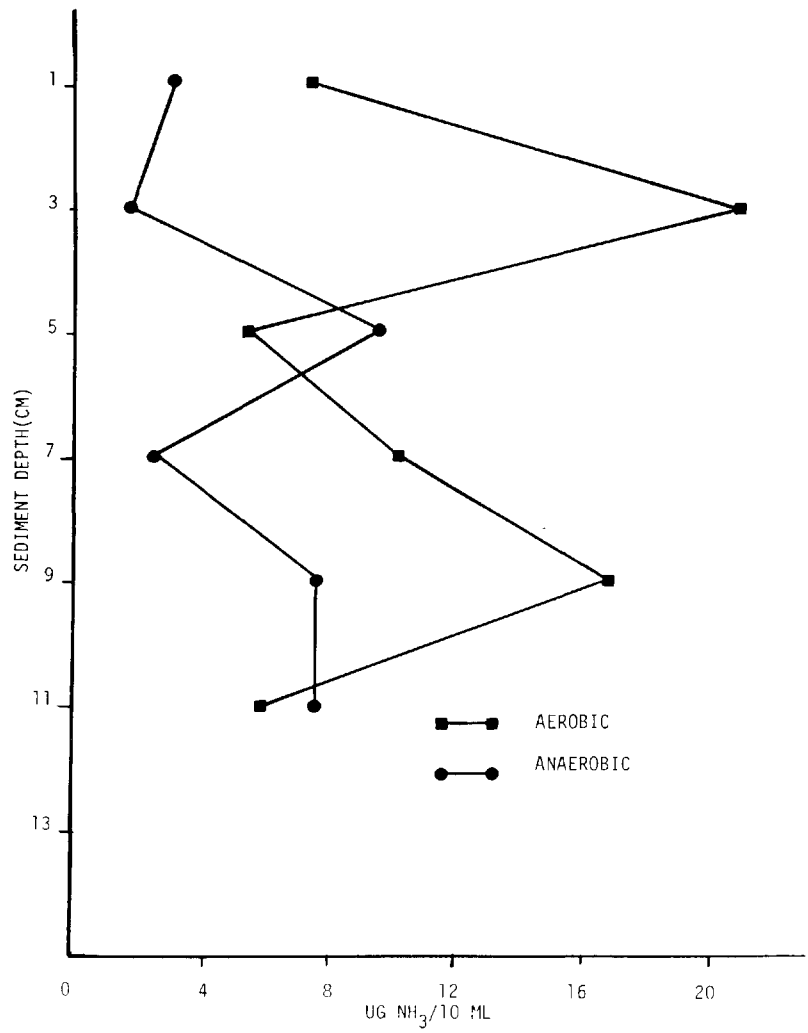


FIGURE 2. Net production of $NH_3$ with inocula from the sediment column after 14 day incubation at 22°C with chitin in mineral salts medium.

22°C and photomicrographs were taken at the sediment-water interface where most rapid colonization took place. An evident background of chitin may be seen in this photomicrograph with clearing observed around particular organisms and colonies. Note the vibrioid bacterial cells associated at the periphery of the cleared area and their general absence from the cleared area (see fig. 4a).

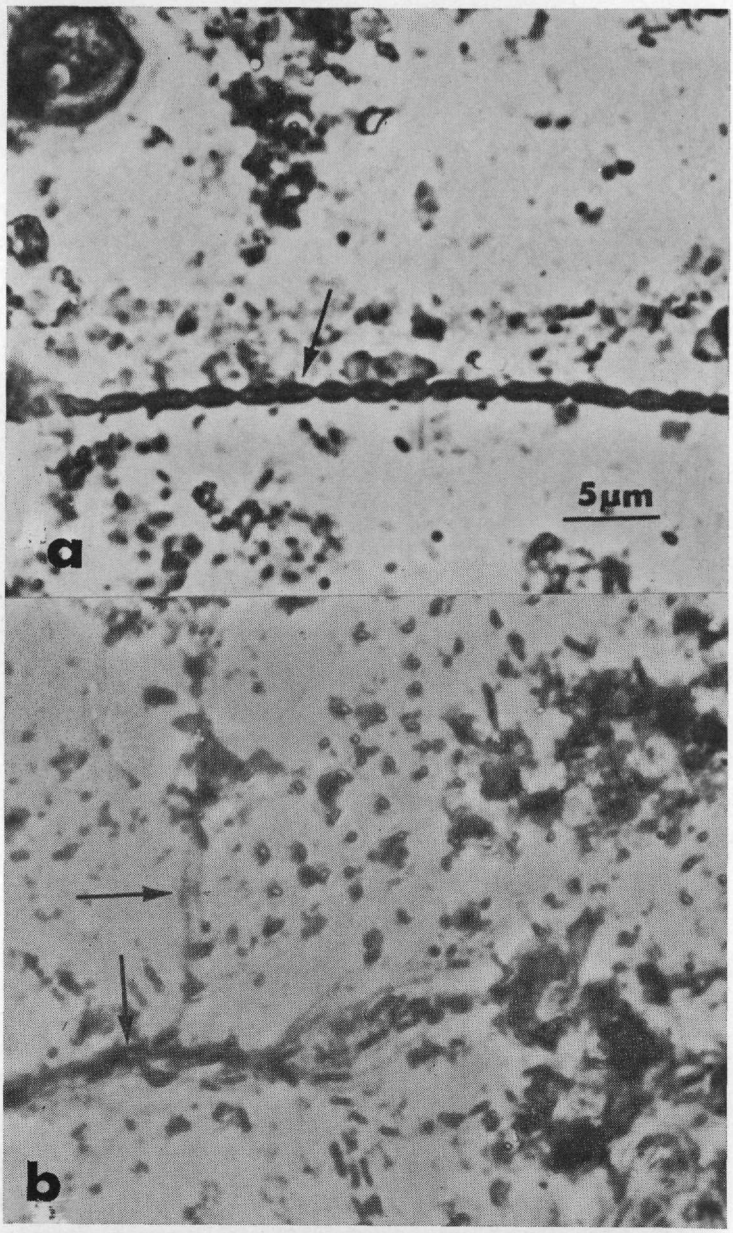


FIGURE 3. Bacteria associated with the control slide at the water-mud interface (a) and within the aerobic zone of the mud column (b) after 7 day incubation. An algal filament is noted by an arrow in (a) while vestiges of a fungal filament (arrows) may be seen in (b). Bar indicates 5 $\mu$m in both photomicrographs.

These types were particularly prevalent in the aerobic zones. Cells typical of the anaerobic zone may be noted in figure 5 (slide incubated for 7 days at 22°C). Cleared areas of suspected hydrolysis are visible with bacteria associated at the periphery.

A succession of microorgaisms was observed to inhabit the chitin coated slides. In the aerobic zone, cocci first became

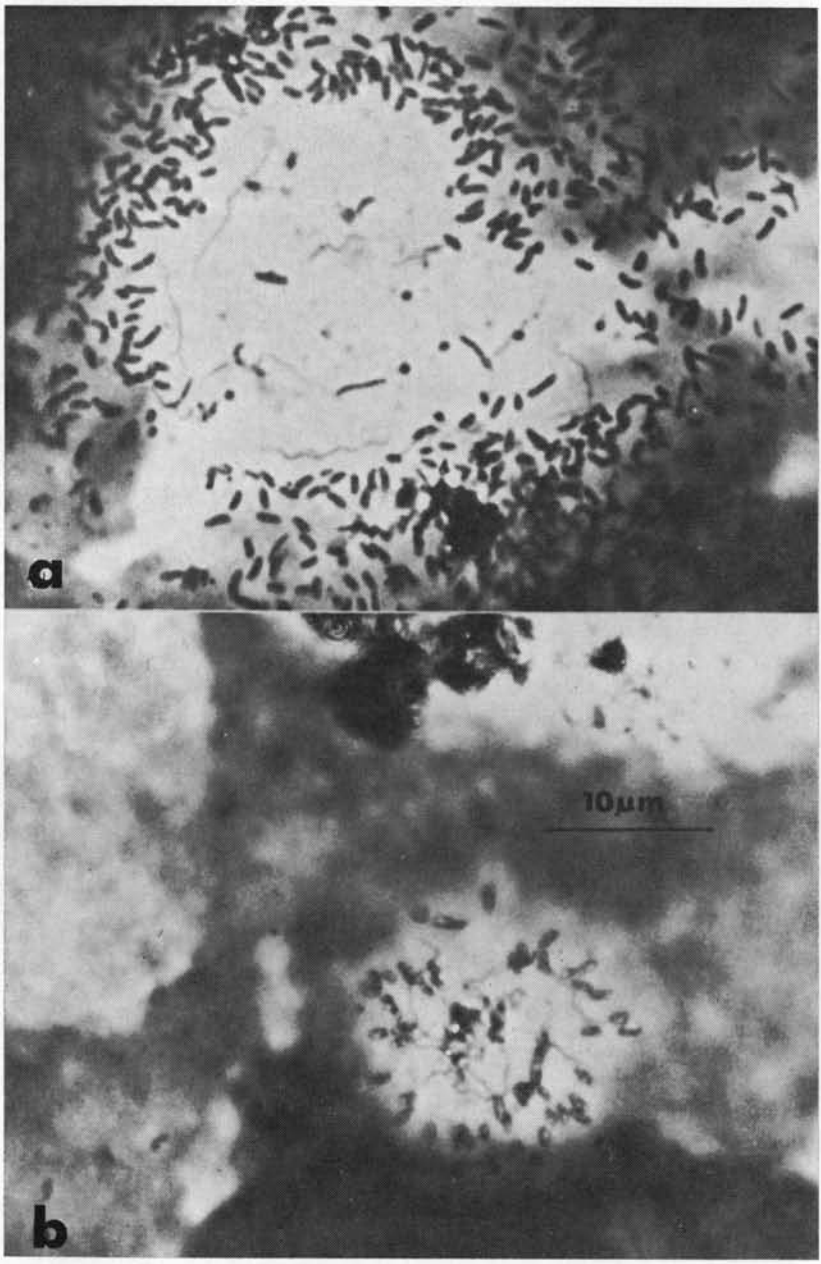


FIGURE 4. Bacterial growth within the aerobic zone of mud sediments associated with a chitin coated slide after 6 day incubation. Rings of hydrolysis may be seen in both (a) and (b). Bar indicates 10 $\mu$m in both photomicrographs.

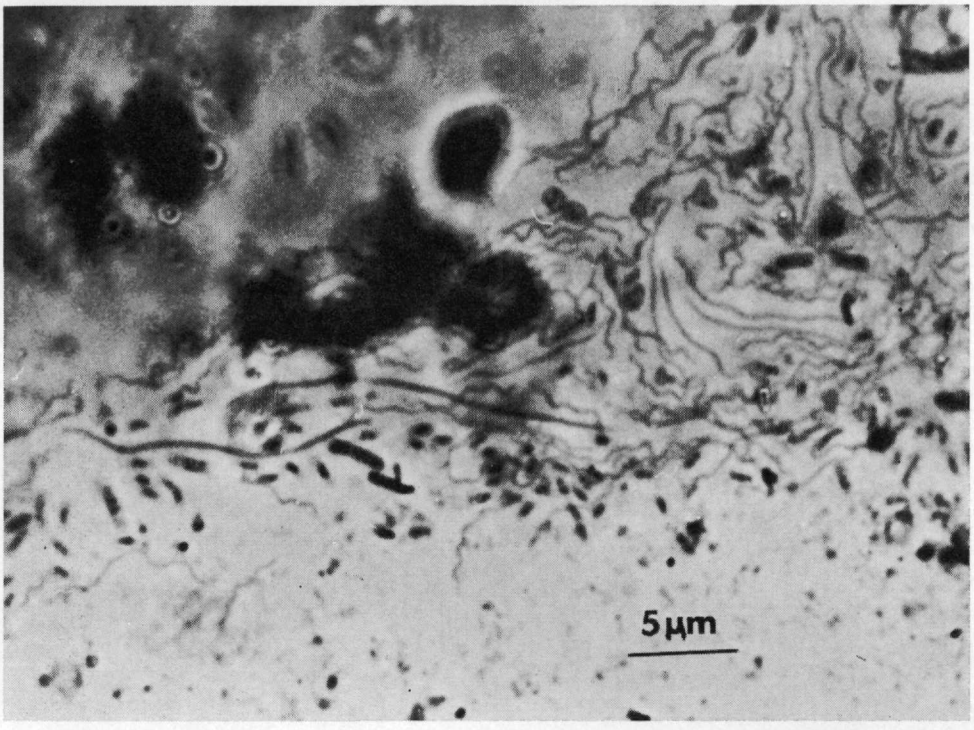


FIGURE 5. Bacteria adhering to chitin coated slide after 7 day incubation at 22°C. Photomicrograph taken at a depth of 4 cm in the mud column.

established but within 7 days gave way to slightly curved rods which predominated until the chitin was digested (fig. 4). In the anaerobic zone, hydrolysis was much slower (10–20 days) with vibrioids dominating the flora at 1 week (fig. 5). After this time spiral and, to a lesser extent, certain vibrioid forms dominated the flora of the slides through the second week. Chitin digestion did not seem to be complete in the anaerobic zone even after 2 weeks of incubation whereas hydrolysis appeared complete in 1 week aerobically.

Sediments from various depths were also examined for presence of chitin (or particulates containing chitin) by the method of Frey (1960). Although quantitative results were not obtained, particulates with chitin content were noted at depths of up to 13 cm in the column.

## DISCUSSION

The similarity of bacterial numbers both aerobically and anaerobically at the various depths may have been due to the freezing of the cores. This procedure may have selected against certain bacterial types while favoring others. Working with cores from the same area, Weeks (1944) had found comparable numbers of heterotrophs both aerobically and anaerobically as in the present study.

The number of chitinolytic organisms reported in this study may be somewhat lower than the actual number active *in situ* because of the absence of other organic material that is known to increase numbers obtained (Hood and Meyers 1973).

The presence of increased $NH_3$ in the supernatants of mud enriched with chitin demonstrated the ability of a sediment bacterial population to hydrolyze chitin. The absence of detectable NAGA may possibly indicate the ability of these same populations to deaminate and/or deacetylate the sugar, therefore yielding no detectable results. This ability has been found in many bacterial isolates by Ortiz *et al* (1972). Enzymatic breakdown of any released NAGA seems very likely in

light of the evidence of chitin disappearance and $NH_3$ build-up found in this case.

The buried slide technique showed the variety of microorganisms involved in chitinolytic activities. The succession of organisms predominating on the slides at different times supported this contention. Similar types were seen in marine sediments by Gray *et al* (1968).

The use of a reprecipitated chitin may be questioned as to whether this would truly indicate chitinolytic organisms in a natural or mixed community of bacteria. Studies such as the one conducted by Sundarraj and Bhat (1972) pointed to the suitability of reprecipitated chitin for bacterial work. Other investigators (Campbell and Williams 1951; Monreal and Reese 1969) have used similar preparations of chitin in studying chitinase systems.

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